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Different Patterns of Cytokine Induction in Cultures of Respiratory Syncytial (RS) Virus-Specific Human T_H Cell Lines Following Stimulation With RS Virus and RS Virus Proteins

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Human peripheral blood mononuclear cell (PBMC) proliferative responses to live respiratory syncytial (RS) virus, formalin-inactivated RS (FI-RS) virus, RS virus F (fusion) protein, and RS virus G (attachment) protein were assessed. All donors responded to challenge with whole RS virus antigens and F and G proteins. F protein responses elicited higher levels of response than equivalent concentrations of G protein in nine out of ten adult RS-seropositive donors. Stimulation of PBMC induced low levels of interleukin 2 (IL-2), interferon γ (IFN-γ), IL-4, and IL-10 production. Human RS virus–specific T cell lines were generated from peripheral blood cultures following in vitro stimulation with RS virus antigens. All lines generated were shown to be MHC class II restricted. Characterisation of the lines was carried out by determining the levels of IL-2, IFN-γ, IL-4, and IL-10 in culture supernatants. T cell lines enriched for RS virus–specific cells provided a more sensitive system than PBMC cultures for the detection of cytokines. The pattern of cytokine production varied for the individual lines, and the detection of T_H1 and T_H2 cytokines was dependent on the nature of the stimulating RS virus antigen. Live RS virus induced a T_H1 pattern of cytokines (IL-2 and IFN-γ), whereas FI-RS virus induced the production of both T_H1 and T_H2 cytokines. In addition, T_H lines specific for individual RS virus proteins produced different cytokine profiles. F protein–specific lines generated T_H1-type cytokines (IL-2 and IFN-γ), whereas G protein–specific lines generated T_H2-type cytokines (IL-4 and IL-10).

KEY WORDS: T cell immunity, T_H1 and T_H2 subsets, viral protein recognition

INTRODUCTION

RS virus is recognised as a major respiratory pathogen of infancy and early childhood, with RS virus–induced bronchiolitis as the major single cause of hospitalisation within this age group. Previous attempts to obtain a suitable vaccine effective against RS virus have been unsuccessful [Gharpure et al., 1969; Wright et al., 1982]. Furthermore, a formalin-inactivated alum-precipitated whole RS virus vaccine not only failed to reduce the incidence of RS virus infection, but actually potentiated severe disease among vaccinees upon subsequent natural infection [Kapikian et al., 1969; Kim et al., 1969]. A more detailed knowledge of the interactions of the virus with the human immune system is therefore an essential prerequisite for the future development of an RS virus vaccine that is both safe and effective.

RS virus has the ability to infect repeatedly and cause disease throughout life, with each infection providing some, although incomplete, protection from subsequent infection [Henderson et al., 1979]. The protective role of anti-RS virus antibodies is well documented. However, despite an accumulated acquisition of resistance to lower respiratory tract disease, reinfections of the upper respiratory tract are common and occur in the presence of high levels of neutralising and complement-fixing antibodies. Less is known about the human cell-mediated immune response to RS virus. RS virus induces MHC class I–restricted cytotoxic T lymphocytes (CTL) that recognise the fusion (F), nucleoprotein (N), and the 22-kDa matrix protein (M_2) as major targets [Cherrie et al.,

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1992]. Study of RS virus–specific MHC class II–restricted T helper (T_H) lymphocyte responses have identified F and, to a lesser extent, P, G, and M_2 proteins, as major targets [Anderson et al., 1991].

The role of the individual components of the immune system in RS virus pathogenesis has been studied in animal models. Studies of BALB/c mice depleted of B cells, $CD4^+$ cells or $CD8^+$ cells have shown that serum antibody can provide complete protection from reinfection and that $CD4^+$ and $CD8^+$ T cells are important for clearing infection [Graham et al., 1991a,b]. Conversely, the same studies found that $CD4^+$ and $CD8^+$ T cells also appear to contribute to illness. Depletion of either $CD4^+$ or $CD8^+$ T cells decreased the severity of disease, whereas depletion of both T cell subsets eliminated illness. This has highlighted the need for a greater understanding of the interaction between the virus and the T lymphocytes *of the host.*

The existence of T_H1 and T_H2 CD4+ T lymphocytes distinguishable on the basis of the patterns of cytokines they produce has been demonstrated in a number of diseases, including leishmaniasis [Heinzel et al., 1989], leprosy [Yamamura et al., 1993], and malaria [Taylor-Robinson et al., 1993]. In these infections, a T_H1 response, characterised by production of IL-2 and IFN-γ, has been associated with control or protection from disease, and a T_H2 response, characterised by production of IL-4 and IL-5, with an inability to control infection.

Recent studies in BALB/c mice have investigated cytokine production following inoculation with various forms of RS virus antigen. Graham et al. [1993] demonstrated that the lungs of BALB/c mice primed with FI-RS virus had higher levels of IL-4 mRNA transcript (T_H2 cytokine) than those primed with live virus, which had a higher level of IFN-γ. They suggested that the T_H2 response may be responsible for the enhanced disease seen in such mice following FI-RS virus immunisation. More recently, Connors et al. [1994] demonstrated that the enhanced pulmonary histopathology induced by RS virus challenge of FI-RS virus–immunised BALB/c mice could be abrogated by the depletion of IL-4 and IL-10 (T_H2 cytokines).

The results obtained so far in animal models provide an important insight into mechanisms of pathogenesis in RS virus disease, but will in the future require extrapolation to human studies. As yet, however, there is no information concerning human T_H subset differential induction by different RS virus antigens. Studies in our laboratory and elsewhere have shown that the F protein is a predominant RS virus antigen with regard to recognition by human $CD4^+$ T helper cells in PBMC cultures [Anderson et al., 1991; Levely et al., 1991]. However, assay for IL-2 production by PBMC in response to RS virus antigens revealed that the response was low and variable [Anderson et al., 1991]. In a subsequent study by Anderson et al., [1993], T_H cell lines were generated from PBMC of RS virus–seropositive donors using inactivated whole RS virus antigen. RS virus–specific $CD4^+$ T cell lines predominantly recognised the F protein, and more consistent levels of IL-2 were detected in the supernatants of the $CD4^+$ T cell line cultures. In this report we describe the human $CD4^+$ T cell memory responses to a range of RS virus and RS virus protein antigens. In addition, the functional characteristics of human RS virus–specific T_H cell lines were investigated with respect to cytokine production in order to identify T_H1 and T_H2 responses.

MATERIALS AND METHODS

Virus

The A2 strain of RS virus was obtained from Dr. E.J. Stott of the Institute of Research on Animal Diseases, Compton, UK. The virus was grown and assayed for infectivity in Hep 2c cells using the fluorescent focus assay [Routledge et al., 1988]. Mock-infected Hep 2c cells were treated in an identical way and used as control antigen.

Preparation of Antigens

RS virus and control Hep antigen were inactivated with formalin (FI; BDH Ltd.) at a final concentration of 1:4,000 for 72 hours at 37°C. The antigens were centrifuged at 50,000 rpm for 1 hour in a Beckman ultracentrifugation rotor. The resultant pellets were resuspended in Eagle's basal medium in Earle's salt solution (Flow Laboratories Ltd.) and adsorbed to alum (4 mg/ml) as described previously [Kim et al., 1969].

The F and G glycoproteins of A2 strain RS virus were purified from infected roller bottle culture lysates of Hep 2c cells by immuno-affinity chromatography. Proteins were eluted in Tris saline (0.01 M Tris-HCl, 0.15 M NaCl, pH 7.4) containing 1% sodium cholate and 6 M NaSCN (pH 7.4) and dialyzed against Tris saline alone for 24 hours at +4°C, as described previously [Routledge et al., 1988]. Control antigen for the purified proteins was RPMI 1640 medium.

Human Donors

Ten RS virus–seropositive adult male and female volunteers were drawn from the staff of the Virology Department of the University of Newcastle upon Tyne. Twenty- to 50-ml samples of peripheral blood were collected from each donor by venipuncture and taken into heparinised tubes with a final concentration of preservative-free calcium heparin of 20 units/ml.

Tissue Typing

The tissue type of adult donors was determined with respect to the major histocompatibility complex (MHC) class II DR locus. Typing was carried out by the Northern Regional Health Authority Blood Transfusion Centre.

Lymphocyte Proliferation Assay

Human PBMC suspensions were prepared from adult peripheral blood upon Ficoll gradients (J Bio). Human lymphocytes were washed and finally resuspended at 1×10^6 cells/ml in RPMI 1640 medium containing 25 mM Hepes buffer and 2 mM L-glutamine (Gibco Ltd.) and supplemented with 10% v/v heat-inactivated autologous plasma (10% RPMI). Two hundred microlitres of

the washed cell suspension was plated into wells of a 96-well plate (Nunclon Ltd.). Ten percent v/v RS virus, RS virus protein, or control antigen was added to the appropriate wells. All antigens were assayed in triplicate for 4, 5, and 6 days and then pulsed with 0.2 μCi ^{3}H-thymidine for 18 hours. Cultures were harvested, and the amount of incorporated ^{3}H-thymidine was detected using a Packard Matrix 96 direct β counter. Stimulation indices of ≥3 were taken to indicate a significant response to antigenic challenge [Anderson et al., 1991].

T Cell Lines

RS virus–specific T cell lines were derived from live RS virus-, FI-RS virus-, F protein-, and G protein-stimulated adult human PBMC by repeated antigen stimulation in vitro. Briefly, PBMC were suspended at 10^6 cells/ml in 10% RPMI 1640 medium, and 2×10^6 cells were plated into wells of a sterile 24-well plate (Costar Ltd.) with 200 μl of antigen. After 7 days, the cells were washed and suspended at 10^6 cells/ml. One millilitre of the washed line suspension was added to each well of a 24-well plate containing 10^6 autologous irradiated PBMC as antigen presenting cells (APCs) and 200 μl of antigen. Ten percent v/v MLA 144 cell supernatant (IL-2 supplement) was added to each well. The restimulation cycle was repeated every 14 days with replacement of 50% of the culture supernatant with 10% RPMI supplemented with MLA 144 supernatant every 2 days.

T Cell Line Proliferation Assay

To measure the proliferative response of cell lines, 4×10^4 cells were cultured in 10% RPMI in 96-well round-bottomed plates (Nunclon Ltd.) with 4×10^5 autologous irradiated APCs in 100 μl/well for 1 to 5 days. One hundred microlitres of live RS virus, FI-RS virus, F protein, G protein, or control antigen was added to appropriate wells. In the last 6 hours of each culture period, 0.5 μCi/well of ^{3}H-thymidine was added to each well, after which time cells were harvested and counted for incorporated ^{3}H-thymidine using a Packard Matrix 96 direct β counter. Stimulation indices of ≥3 were taken to indicate a significant response to antigenic challenge [Anderson et al., 1991].

MHC Class I and II Restriction Studies

The proliferative responses of all lines were studied in the presence or absence of anti-human MHC class I antibody (Sera-lab Ltd.) or anti-human MHC class II antibody (Serotech Ltd.) at a final concentration of 10 μg/ml. The percentage inhibition of proliferation due to the presence of antibody was calculated.

Cytokine Assays

Cell-free supernatants from PBMC assays and line cultures were removed and assayed for cytokines. Multiple dilutions of supernatants from cultures were tested. Results presented in this report represent the maximum levels of cytokines found.

IL-2 Assay

IL-2 dependent CTLL cells [Gillis and Smith, 1977] were maintained in RPMI 1640 medium supplemented with 10% v/v heat-inactivated foetal calf serum (HIFCS), 0.05 M 2-mercaptoethanol (2-Me), and L-glutamine (2 mM), with 200 IU/ml recombinant IL-2. The assay was performed by washing the CTLL cells three times with medium and suspending them at 5×10^4 cells/ml. One hundred microlitres of this suspension was incubated in 96-well round-bottomed microtitre plates with 100 μl of diluted test samples for 20 hours before the addition of 0.5 μCi/well of ^{3}H-thymidine for 4 hours. The cells were harvested and counted for incorporated ^{3}H-thymidine. The results were calculated as the geometric mean counts per minute (cpm) of triplicates after subtraction of background cpm (CTLL cells incubated with medium only). In each assay, wells containing a series of dilutions of recombinant IL-2 acted as positive controls.

Selected supernatants were also tested in the presence or absence of anti-human IL-2 antibody (British Biotechnology Ltd.) Incubation of supernatants in the presence of anti-human IL-2 at a concentration of 10 μg/ml was found to block proliferation of the CTLL cells.

IL-4 Assay

The IL-4 dependent cell line CT.h4S (kindly supplied by Prof. W.E. Paul, NIH, Bethesda, MD) was maintained in RPMI 1640 medium supplemented with 10% v/v HIFCS, 0.05 M 2-Me, 2 mM L-glutamine, 1 mM sodium pyruvate, and recombinant IL-4 (10ng/ml). The assay was performed as described above by washing the CT.h4S cells with medium and suspending the cells at 1×10^5 cells/ml. Fifty microlitres of this suspension was incubated in 96-well microtitre plates with 50 μl of diluted test samples and 12.5 μg/ml anti-human IL-2 antibody for 48 hours before the addition of 0.5 μCi/well of ^{3}H-thymidine for 4 hours. After this time, cells were harvested and counted for incorporated ^{3}H-thymidine. The results were calculated as the geometric mean counts per minute (cpm) of triplicates after subtraction of background cpm (CT.h4S cells incubated with medium alone). In each assay, wells containing a series of dilutions of recombinant IL-4 acted as positive controls. Monoclonal antibody (MAb) inhibition studies were performed as above confirming that activity could be ascribed to IL-4.

IL-10 and IFN-γ Assays

Commercial enzyme-linked immunosorbent assay (ELISA) kits (Medgenix Diagnostics) were used to assay culture supernatants for IL-10 and IFN-γ.

RESULTS

PBMC Responses to In Vitro Challenge With RS Virus Antigens

The proliferative response of adult human PBMC to challenge with whole RS virus and RS virus F (fusion) and G (attachment) protein was examined. Optimum proliferative responses to intact virus were consistently

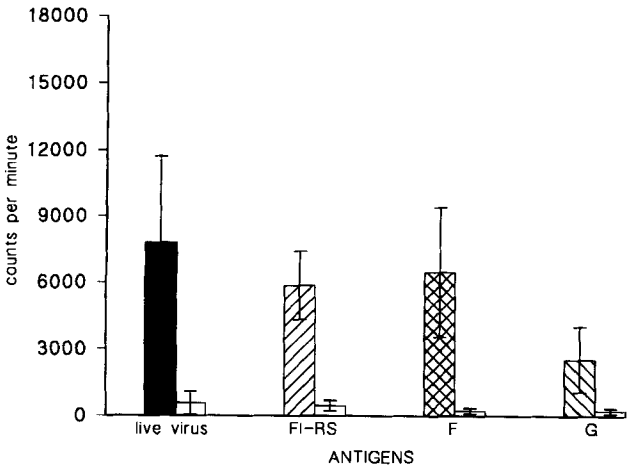

Fig. 1. Mean proliferative responses of PBMC from ten adult human donors in response to live RS, FI-RS, F protein, G protein, and control antigens. Responses were measured by ^{3}H-thymidine incorporation in counts per minute. Results are expressed as geometric mean of triplicate cultures ± S.D.

observed at a multiplicity of infection of 0.1. The optimum response to challenge with the two polypeptide preparations was observed at concentrations of 2.5 μg/ml. Maximum proliferation of cells was found on day 5, decreasing thereafter.

The mean PBMC proliferative responses of ten adult human RS virus seropositive donors to challenge with RS virus antigens are presented in Figure 1. PBMC from all ten donors responsed (S.I. > 3.0) to challenge with live and FI-RS whole virus antigen preparations. All donors responded to F and G protein challenge, with F protein–specific responses being the highest observed in nine out of ten donors and of a similar magnitude to those induced by live virus, despite the wide variety of HLA types in the donor population. PBMC from one of the ten donors demonstrated a proliferative response to G protein of equal magnitude to F protein. Challenge with RS virus control antigen did not induce a proliferative response in excess of that recorded in the medium only controls.

PBMC culture supernatants from the adult human donors were examined for the production of IL-2, IFN-γ, IL-4, and IL-10 in response to antigenic challenge. Culture supernatants were collected daily from PBMC cultures stimulated with challenge antigen. Cytokines were detectable in supernatants between day 1 and day 5 post-stimulation, with peak cytokine production on day 3 or 4. Varying levels of cytokines were detectable in supernatants from all donors. The mean levels of IL-2, IFN-γ, IL-4, and IL-10 produced at day 3–4 following antigenic stimulation of PBMC are presented in Figure 2.

Live virus stimulation of PBMC induced high levels of IL-2 and IFN-γ production, but little IL-4 and IL-10, whereas FI-RS virus stimulation induced lower levels of IL-2 and IFN-γ than live virus, but more IL-4 and IL-10. F protein stimulated PBMC produced higher levels of IL-2 and IFN-γ than G protein, but less IL-4 and IL-10.

Characterisation of RS Virus Antigen-Specific T Cell Lines

Human T cell lines were generated by in vitro stimulation of PBMC from five adult human RS virus seropositive donors with RS virus antigens. Figure 3 demonstrates the kinetics of the proliferative response of a representative line to challenge with antigen. Maximum proliferation of lines was found on day 4, decreasing thereafter. Challenge of the lines with control antigens induced only minimal proliferation.

Lines were generated to live virus, FI-RS virus, F protein, and G protein. Lines proliferated in response to their stimulating antigen and also to other RS virus antigens (Fig. 4). All lines generated to live virus proliferated to a lesser extent to FI-RS virus challenge, and vice versa. All F protein lines responded well to the stimulating antigen and not to challenge with G protein. Similarly, G protein lines responded to G protein but did not proliferate specifically in response to F protein. Responses of the lines to control antigens were minimal. MHC class I and II restriction studies showed that the proliferative responses of live RS virus, FI-RS virus, F protein, and G protein T cell lines were inhibited by the addition of anti-human MHC class II antibody (94.4 ± 0.8% to 98.9 ± 0.8% inhibition) but not by anti-human MHC class I antibody (<2% inhibition of proliferation), demonstrating the presence of MHC class II–restricted cells in all lines generated.

Line culture supernatants were assayed for the production of IL-2, IFN-γ, IL-4, and IL-10. Cytokines were detectable in supernatants between day 1 and day 4 post-stimulation, with peak cytokine production on day 2 or 3. The peak levels of cytokines produced by the lines are presented in Figure 5. Assay of supernatants from the live virus lines demonstrated the production of IL-2 and IFN-γ, but no IL-4 or IL-10 upon antigenic challenge with live RS virus. In contrast, the FI-RS virus lines stimulated with the homologous antigen produced IL-2, IL-4, IL-10, and low levels of IFN-γ.

The cytokine profile of the F protein lines was similar to that of the live virus lines, with production of IL-2 and IFN-γ, in the absence of IL-4 and IL-10 on challenge with F protein. In contrast, the G protein lines produced IL-4, IL-10, and a low level of IL-2, but no IFN-γ on challenge with G protein. Challenge of the T cell lines with the control antigens did not induce detectable levels of the four cytokines (data not shown).

DISCUSSION

A substantial body of data has suggested a segregation of murine CD4 T cells into at least two subsets, designated T_H1 and T_H2, based primarily on specific patterns of cytokine secretion. T_H1 cells have been shown to produce IL-2, IFN-γ, and TNFβ and promote delayed type hypersensitivity (DTH) reactions. In contrast, T_H2 cells produce IL-4, IL-5, IL-6 and IL-10, promoting B cell activation and antibody production, particularly of the

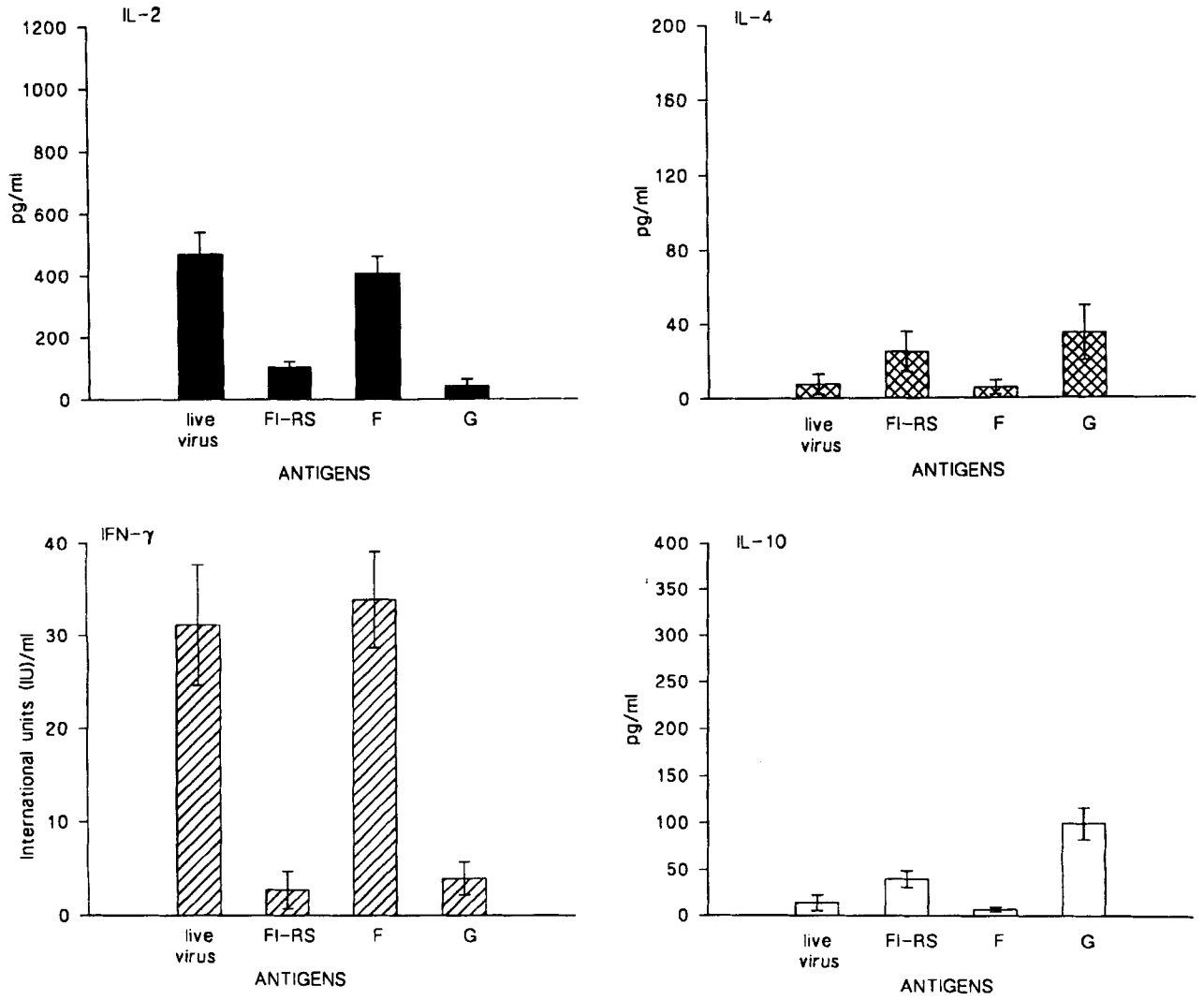

Fig. 2. Mean levels of IL-2, IFN-γ, IL-4, and IL-10 production by adult human donors at day 3–4 following challenge of PBMC with whole RS virus antigens and F and G proteins. Levels are expressed as pg/ml or IU/ml ± SD.

IgE and IgG1 isotypes [Mosmann and Coffman, 1989]. A similar T_H cell dichotomy is thought to exist in humans [Romagnani, 1991], although some cytokines, such as IL-10, may not be as restricted as they are in the murine system [Del Prete et al., 1993]. The preferential induction of T_H cell subsets may be relevant to in vivo responses in human viral infections, such as human immunodeficiency virus (HIV) and measles virus. During HIV infection, a switch from a T_H1-type response to a T_H2-type response is thought to occur since the progression to acquired immunodeficiency syndrome (AIDS) is characterised by loss of IL-2 and IFN-γ production concomitant with increases in IL-4 and IL-10 production [Clerici and Shearer, 1993]. Analysis of cytokine production by measles virus vaccine recipients suggests that T_H2 cells producing IL-4 are preferentially activated by the measles virus vaccine [Ward and Griffen, 1993].

A previous study by Graham et al. [1993] demonstrated relative increases in IL-4 mRNA expression following priming of BALB/c mice with FI-RS virus and challenge with live virus, compared to a decrease in IL-4 mRNA and an increase in IFN-γ mRNA expression following live virus priming and challenge. In addition, Connors et al. [1992] demonstrated that depletion of CD4+ T cells abrogated the enhanced pulmonary pathology associated with FI-RS virus priming of BALB/c mice.

Other murine studies [Alwan et al., 1993] have shown that T cell lines derived from vaccinia RS virus F recombinant primed BALB/c mice secreted IL-2 and IL-3, but not IL-4 or IL-5, which is a T_H1-like pattern of cytokine production. By contrast, RS virus G protein–specific T cell lines produced IL-3, IL-4, IL-5, and a small amount of IL-2, which is more consistent with a T_H2-type response.

Conflicting results were obtained in a study by Graham et al. [1993] in that priming of BALB/c mice with subunit F protein induced a T_H2 pattern, rather than a T_H1 pattern of cytokine mRNA expression as characterised by an increase in IL-4 mRNA and a decrease in

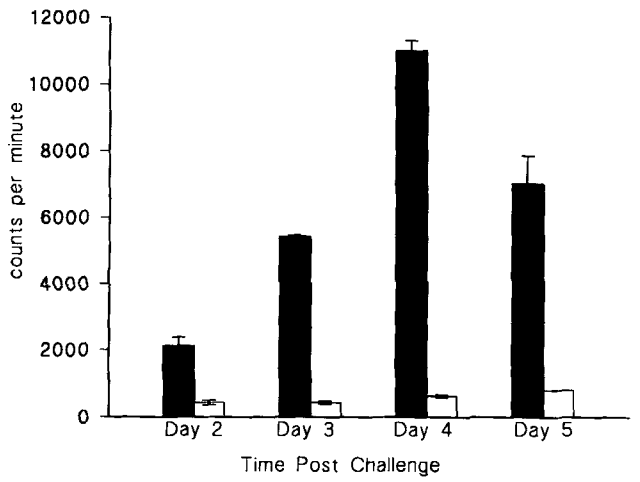

Fig. 3. Kinetics of the proliferative response of a representative RS virus antigen-specific T_H cell line, where solid bars show the response to RS antigen and open bars show response to the antigen control. Results are expressed as the geometric mean of triplicates ± S.D.

IFN-γ mRNA. However, the F protein preparation used in the above study was adsorbed to alum, an adjuvant which is a potent inducer of IgE responses [Allison and Byars, 1991] and which is thought to stimulate cells of the T_H2 subtype.

The different patterns of cytokine production by the F and G protein-specific T cell lines, demonstrated by Alwan et al. [1993], may explain the different types of immunopathology seen following priming of mice with F or G protein. Mice primed with F protein develop lung haemorrhage and neutrophilic pneumonitis, whereas G protein priming induces an intense pulmonary eosinophilia [Openshaw et al., 1992]. Furthermore, this pulmonary disease was shown to occur when RS virus-infected mice received G protein-specific cells which were characterised as T_H2, whilst transfer of F protein specific cells, characterised as T_H1, caused only minimal enhancement of pathology [Alwan et al., 1994]. Furthermore, a study by Bright et al. [1995] demonstrated that in vitro RS virus stimulation of lymph node cells from RS virus-primed BALB/c mice resulted in a switch from IL-2 to IL-4-producing cells by 6 days of culture. In contrast, F

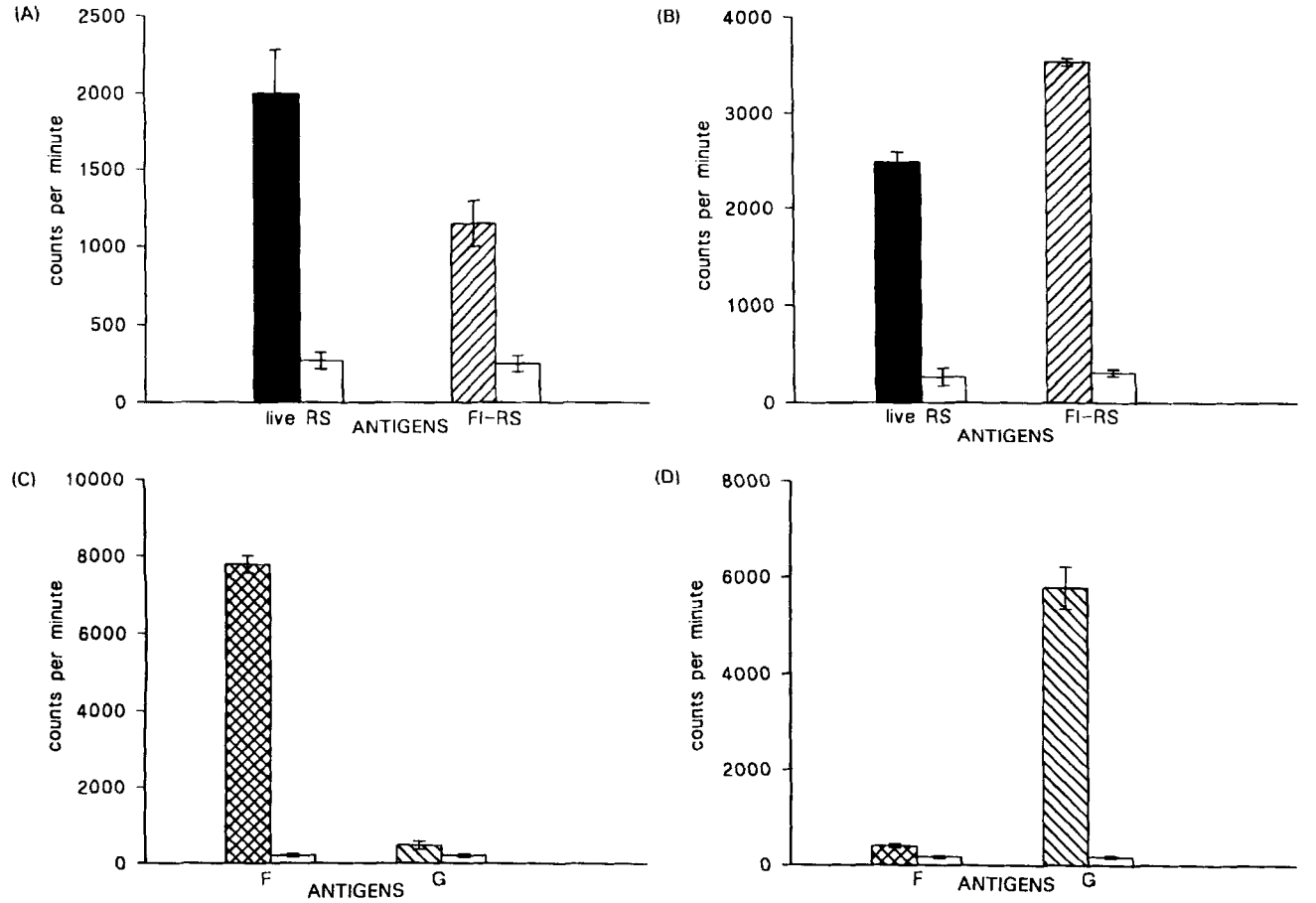

Fig. 4. Day 4 proliferative responses of representative **(A)** live RS virus, **(B)** FI-RS virus, **(C)** F protein, and **(D)** G protein T cell lines following challenge with live virus, FI-RS virus, F protein, G protein, and control. Results are expressed as the geometric mean of triplicates ± S.D.

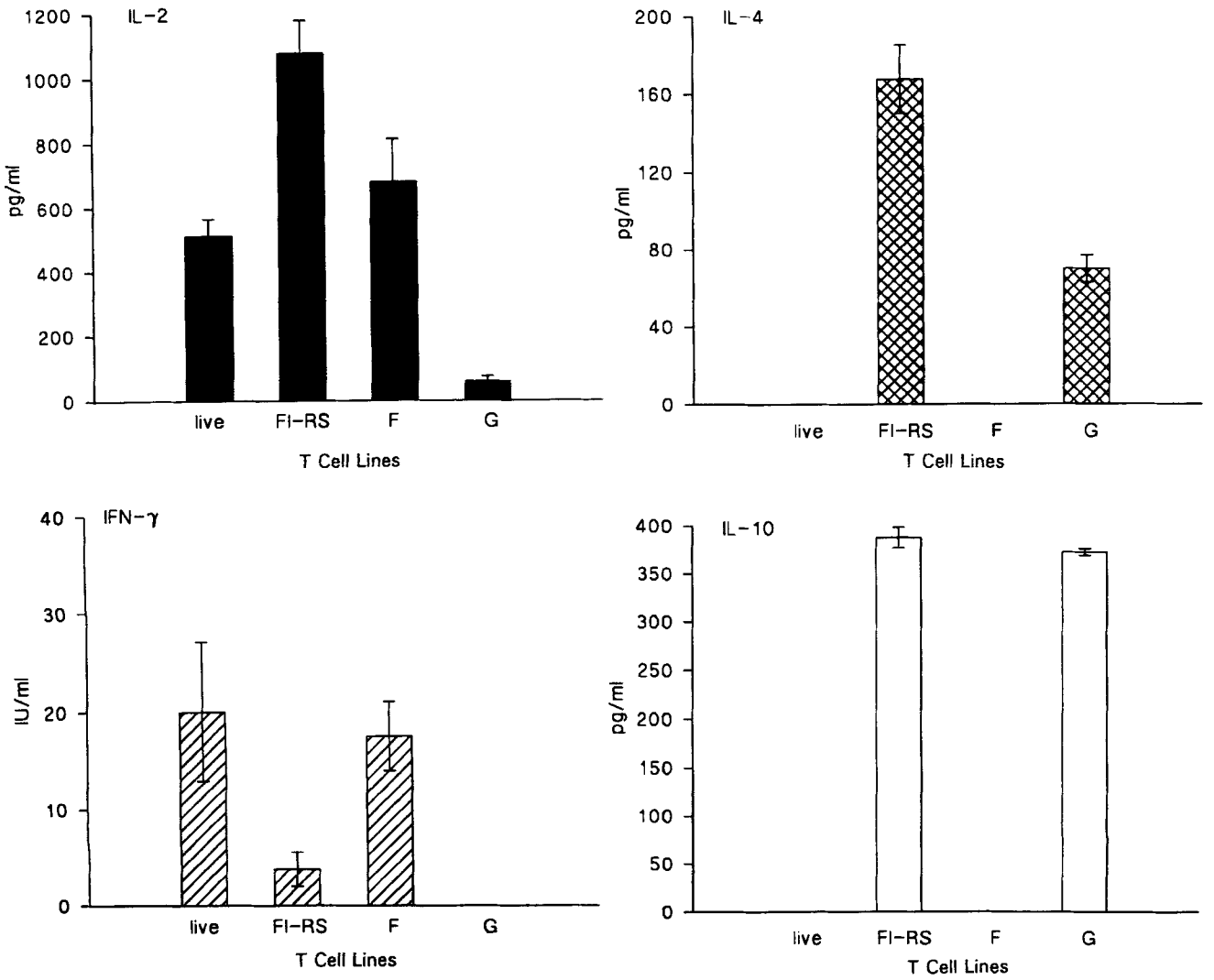

Fig. 5. IL-2, IFN-γ, IL-4, and IL-10 production by human live, FI, F, and G RS virus antigen–specific T cell lines at day 2–3 following challenge with the homologous RS virus antigens. Results are expressed as the mean of five line cultures ± S.D.

protein stimulation of F protein–primed mice induced secretion of IL-2 throughout the culture period, again suggesting that different vaccine preparations can induce distinct patterns of immune response.

In the present study, PBMC and RS virus antigen–specific T cell lines were characterised by determining the levels of antigen induced IL-2, IFN-γ, IL-4, and IL-10 in culture supernatants. The levels of cytokines detected in PBMC supernatants were generally low. However, IL-2, IFN-γ, IL-4, and IL-10 were present in RS virus antigen–stimulated PBMC cultures from all donors tested, suggesting a T_H0 pattern or a mixed T_H1/T_H2 pattern of cytokine production. The generation of T cell lines enriched for RS virus antigen–specific cells provided a more sensitive system for the detection of cytokines, with higher levels of IL-2, IL-4, and IL-10 being detected. Higher levels of IFN-γ were detected in PBMC cultures rather than in line cultures which may be due to stimulation of cells, such as natural killer (NK) cells, which are also able to secrete IFN-γ and are present in the PBMC cultures. The pattern of cytokine production by the lines varied and was dependent on the nature of the stimulating RS virus antigen. The cytokine profile of the live RS virus stimulated T cell line (IL-2 and IFN-γ) was consistent with a T_H1-type response. In contrast, the FI-RS virus line produced both T_H1 and T_H2 cytokines. In vitro stimulation of T cells with either F or G glycoprotein also induced distinct patterns of cytokine production, with a T_H1-type response associated with F protein stimulation and a T_H2-type response associated with G protein stimulation. This study represents the first demonstration of differential induction of cytokines in human T_H cell lines following stimulation with different RS virus antigens.

Anderson et al. [1994] examined cytokine responses to RS virus in human PBMC cultures from adults, RS virus–seropositive children, and RS virus–seronegative children. This study failed to detect secreted IL-2 and

IFN-γ from RS virus–stimulated PBMC, although cytokine mRNA was detectable using a semiquantitative polymerase chain reaction (PCR). PBMC from all three groups of patients showed predominantly RS virus–specific increases in IL-2 and IFN-γ mRNA, although some patients also responded with an increase in IL-5 mRNA but none with an increase in IL-4 mRNA. The predominance of IL-2 and IFN-γ and the absence of IL-4 mRNA expression following live RS virus stimulation may suggest that naturally acquired RS virus induces a T_H1 memory T cell response. However, the importance of IL-5 mRNA expression detected in a number of children in the study was not clear.

Induction of T_H1 cells demonstrated in the present study in response to live virus might be expected to be beneficial in eliminating a virus infection through the secretion of IFN-γ to activate macrophages and cytotoxic T cells. By contrast, production of IL-4 and only low levels of IFN-γ in response to the FI-RS virus may be important in determining the process of enhanced disease in FI-RS virus–vaccinated individuals following subsequent natural infection. Post-mortem examinations carried out on children who died following vaccination with FI-RS virus showed diverse pathological changes which included eosinophil infiltration in the lungs [Kim et al., 1969]. Furthermore, the appearance of eosinophils in the lungs of mice primed with G protein [Alwan et al., 1994] may also be relevant to the pathogenesis of RS virus disease in humans. It has been suggested that children who recover from bronchiolitis have an increased incidence of asthmatic symptoms many years after the event [Welliver et al., 1979]. Eosinophils have been demonstrated to be intimately associated with asthma [Frigas and Gleich, 1986], and Robinson et al. [1992] have detected a T_H2-like cytokine mRNA expression profile in bronchoalveolar samples of atopic asthmatics.

The precise role of $CD4^+$ T cells in human RS virus disease remains difficult to determine. Although the studies reported here were carried out in adult human peripheral blood and not in the human lung, we provide evidence of the differential production of T_H1 or T_H2-type cytokines depending on the nature of the RS virus antigenic stimulus. The distinct T cell subsets of the immune response induced by different antigenic preparations may have an important bearing on the design of future RS virus vaccines.

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